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Art. I.—*An Investigation of the Bacterial Pollution of the Waters of Port Phillip Bay with Special Reference to the Effluents from the Melbourne and Metropolitan Sewage Farm near Werribee.*

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I. Introduction.

The following investigation was undertaken in order to determine the extent of bacterial pollution of Port Phillip Bay. Although this bay contains a large volume of water it is almost entirely landlocked. Melbourne is situated at a point farthest from the open ocean, and through it flows the River Yarra, which receives much of the surface drainage of the city and some industrial effluents; this river, as well as some large city and suburban drains, discharges directly into the bay within the city boundaries. The Melbourne sewage is treated by a method of broad irrigation at the Melbourne and Metropolitan Sewage Farm situated near Werribee, 20 miles from Melbourne, and this report is concerned primarily with the effluents which are discharged therefrom into the bay. Examination of these over a period of years has shown that chemically they are suitable for discharge, but bacteriological investigations both quantitative and qualitative, though showing a marked improvement in the effluents as compared with the sewage as it enters the farm, indicate the desirability of definitely establishing the fact that final discharge of the effluents into the bay is a safe and effective method of disposal. This investigation has been concerned with the effect of the effluents not only in the immediate vicinity of Werribee, but also at more remote parts of the bay, such as Geelong (45 miles from Melbourne), and has included also an examination of samples collected near Melbourne at the entry to the bay of the River Yarra and several large drains.

II. Details of Bacteriological Investigation.

In the bacteriological examination of samples of water for domestic purposes, the accepted practice is the determination of total bacterial content and the number of *B. coli* per cubic centimetre. Though the total count gives valuable information, the *B. coli* content is probably the more important from the point of view of public health. This organism is the commonest inhabitant of the intestine of human beings and animals, so that its presence in water definitely indicates pollution with material from such sources. When *B. coli* is found in water in relatively large numbers, the possibility must be recognized that disease-producing organisms, such as the typhoid bacillus, may also

be present. As organisms of the latter type are not usually very numerous, they are not so easily detected by routine bacteriological methods as *B. coli*, and as this organism is always associated with them in large numbers, it can be taken as a suitable indicator of danger. If the *B. coli* content of a water sample is within certain low limits, it is reasonable to say that such water conforms to a suitable standard of purity.

The technique recommended in standard methods for the bacteriological examination of a drinking water was used, plate counts being made on nutrient gelatine and agar and the presumptive test for *B. coli* carried out in neutral-red lactose peptone water with quantities of the sample to be tested varying from 50 c.c. to 0.00001 c.c.

Confirmatory tests for *B. coli* were performed with pure cultures isolated on MacConkey(9) plates from positive "presumptive" tubes. Numerous confirmatory tests are available, but they are not all of equal differential value. Those selected by Houston(6), the well-known "flaginac" reactions, are still in current use, though many workers have preferred various other series of tests, and in the present investigation it was decided to use those which give more definite evidence regarding the origin of the lactose fermenters in question, distinguishing true *B. coli* more easily from the *Aerobacter* group, which is generally of soil origin, and may be confused with *B. coli* on the results of the "flaginac" tests.

The confirmatory tests used here, typical lactose fermentation having been shown, were the citrate test, the methyl red test, and the indol test.

The citrate test shows whether an organism can utilize a citrate as its only source of carbon. In Koser's(7) liquid citrate medium, organisms of the *B. coli* group do not grow sufficiently to produce a visible turbidity, while *Aerobacter* organisms grow readily and produce a marked turbidity. These latter organisms in their utilization of the citrate produce alkaline end-products which can be detected by an indicator. Simmons'(12) citrate agar medium takes advantage of this fact, citrate utilization being indicated by a change in colour of the medium. Plate cultures of this medium were used in preference to fluid cultures not only because the colour change can be observed more easily than the production of turbidity, but also because the solid nature of the citrate agar facilitates the detection of impure cultures.

The plates were inoculated from fluid cultures of the various organisms, incubated at 37° C. and examined in 24 and 48 hours. The results obtained after the longer incubation period were always the same as those for the short period, so that 48 hours

incubation seemed unnecessary. A few of the cultures studied gave an indefinite reaction on citrate agar, and were tested further in liquid citrate medium where a positive result was observed almost invariably.

The methyl red test (2) again differentiates between the *B. coli* and *Acrobacter* groups, and depends upon differenees in carbohydrate metabolism. The standard glueose peptone medium was used, and a minimum incubation period of five days was found necessary for sharp differentiation between positive and negative reaetions.

The Vosges and Proskauer reaction was not used as it eorrelates almost exaetly with the methyl red test and gives less reliable results.

With regard to the indol test all true *B. coli* should produce indol, while generally *Aerobacter* group organisms do not. However, there are exceptions to both these rules, so that the diagnostic value of the indol test alone is very small. Nevertheless, it was performed throughout the investigation, with peptone water cultures incubated for seven days at 37° C. and tested with the Ehrlich-Boehme reagent.

Throughout all the tests there was extremely good correlation between the citrate test and the methyl red test. The greater number of organisms corresponded to the *B. coli* type, being citrate negative, methyl-red positive, while the great majority of the others were eitrate positive, methyl-red negative. With regard to the indol test a positive reaction was obtained with most of the organisms of the *B. coli* group, but a few members of the *Aerobacter* group also gave the reaetion; for this reason production of indol alone was considered not to be an adequate differential test, and where this result was at variance with the eombined results of the other two tests it was disregarded. With these three tests other workers (1, 5) have obtained the same correlation between the tests themselves and also between these tests and the habitat and sanitary significance of the organism. In the present investigation the latter was also the case, as almost all lactose fermenters from uncontaminated sea water, such as ocean water, were citrate positive, methyl-red negative, indol negative, corresponding to the non-faecal type of whieh the sanitary signifieance is very slight.

The Dominick Lauter medium (3, 8), devised for detecting *B. coli* in water, was tested in conjunction with the full confirmatory tests throughout the investigation, but generally speaking the results were disappointing, for although this medium eliminated false positive reactions due to anaerobes and symbiotic combinations, most organisms of the *Aerobacter* group gave the same reaction as *B. coli*.

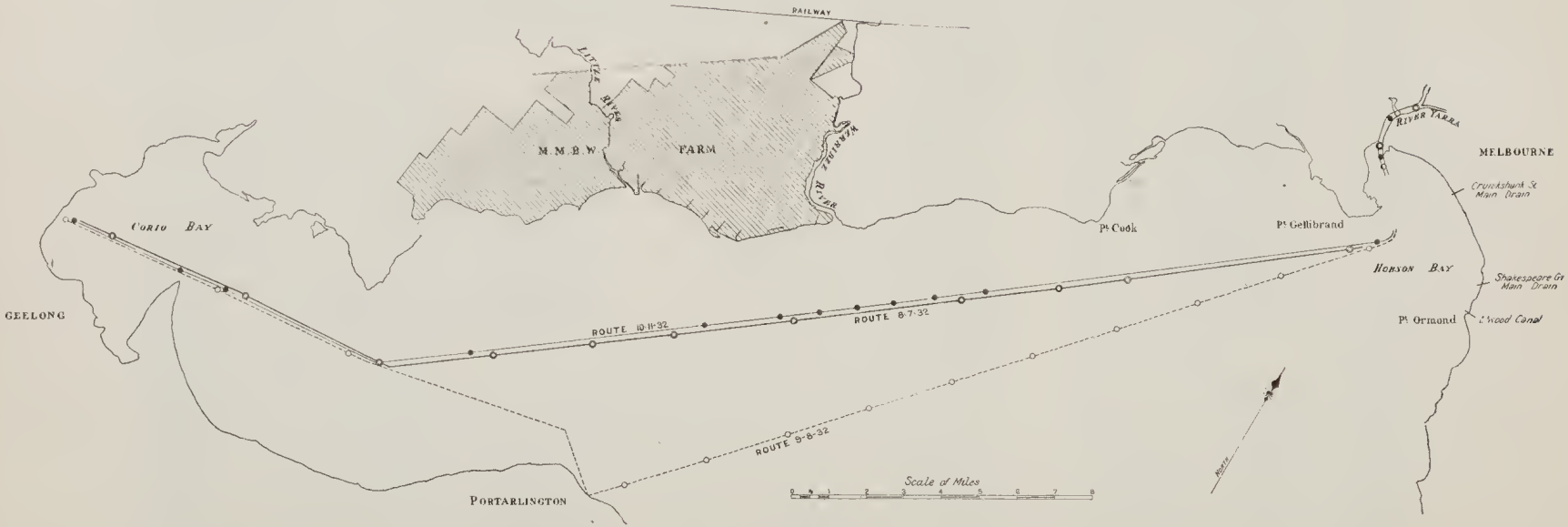

Fig. 1. Port Phillip Bay between Melbourne and Geelong, showing the position of sampling points on each of three routes.

III. Description of Selected Water Samples.

In this investigation the effluents from the farm discharging into the waters of Port Phillip Bay were found to contain approximately 1,000,000 bacteria per 1 c.c., including about 10,000 *B. coli*, some of which may originate from the cattle pastured on the farm. Therefore, in order to determine how quickly dilution renders these effluents innocuous it was necessary to establish, first of all, the bacteriological state of uncontaminated sea water, and note the transition from a state of pollution at the point of entry of the effluents into the bay to one of original purity of sea water at various distances from this point.

The following samples were examined:—

1. *Ocean Water.*—In order to obtain a standard for the bacterial content of pure sea water with which contaminated water might be compared, samples of deep sea water from the ocean in Bass Strait off Cape Schanck were examined.

2. *Bay Water.*—Samples were collected in Port Phillip Bay at various points between Melbourne and Geelong. Two trips were made on the s.s. *Edina* and one on the s.s. *Sphene*, the course on 8.7.32 and 10.11.32 being within 2 miles of the foreshore of the Metropolitan Farm where effluents are discharged. (Fig. 1.) The course on 9.8.32 extended from Melbourne to Portarlington and thence to Geelong, and is shown by the dotted line on Fig. 1. On this trip the samples that were collected were taken at least 5 or 6 miles from the farm.

3. *Bay Water off Shore at Werribee.*—For more detailed investigation it was decided to take samples of sea water at the farm close inshore near the mouths of the effluent drains. For this purpose various points were chosen just off the foreshore and permanently marked with numbered buoys. There were three series labelled A, B, and C, each extending across the front of the farm, commencing at Little River, the maximum distance from the surveyed shore line being 100, 400, and 800 yards for the A, B, and C series respectively. The actual shore line of the farm, however, is very ill-defined, and moreover these distances vary with the tide. There were seven sampling points in each of the A and B series and nine in the C series, C1 and C9 being as close inshore as the A stations. The samples were collected from a motor boat, and ten trips were made under varying conditions of tide, weather, and current during the

months of September, October, and November, 1932. Fig. 2 illustrates the foreshore of the farm, and shows the locations of the various sampling points and of the six main effluent drains marked as 165W, 105W, 25W, 15E, 55E, 95E. It will be noted that most of the A series of points are opposite the mouth of a drain, and should therefore show the maximum amount of pollution.

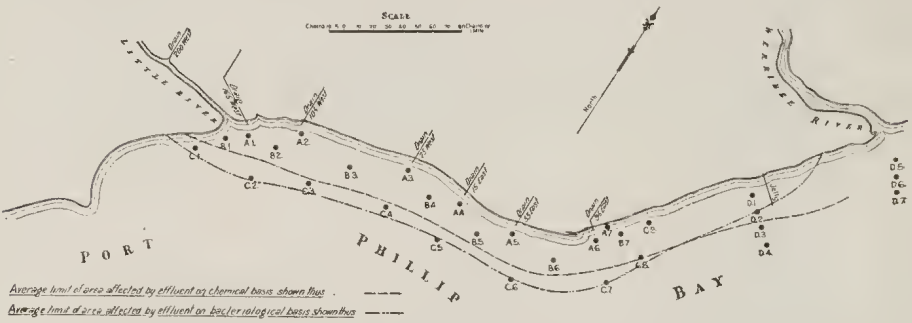

Fig. 2. Foreshore of Metropolitan Sewage Farm, showing position of buoys, sampling points and main effluent drains, together with the average limit of area affected by effluents.

After the first five trips had been made an examination of the results indicated the desirability of examining additional samples of water further to the east. Accordingly, seven more buoys were set up and labelled D1 to D7. D1, D2, D3, and D4 were placed near the farm jetty, D1 being about 100 yards from the shore, D4 about 800 yards from the shore, and the other two equally spaced between them. D5, D6, and D7 were placed near the mouth of the Werribee River, D7 being the farthest from the shore.

Although the general character of the farm effluents was well known from a long series of monthly tests made in this laboratory during 1930 and 1931, and from tests which are still being made regularly every three months, it was decided that the investigation would profit by a knowledge of the exact bacteriological condition of the six effluent drains shown in Fig. 2 at the time of collection of the other samples. These further tests entailed the collection, on each trip to Werribee, of water samples from 36 different points including the six effluent drains. In addition to the bacteriological examination, chemical analyses for chlorine content were carried out on all these samples in order to determine the proportion of sea water to fresh water in each sample, i.e., the rate of dilution of the effluents in the sea, and also to indicate the direction of any constant natural drift. With reference to the latter these chlorine figures always seemed to indicate a drift in an easterly direction parallel to the shore line, while drifts in various directions, probably temporary surface or local currents, were indicated by fluorescein thrown

into the water. The direction of the drift given by the fluorescein test, together with tide, wind, and general weather conditions, times of sampling, and the state of the water, was recorded on each trip.

4. *Drain Water.*—It is obvious that there are many other actual and potential sources of pollution of the waters of Port Phillip Bay besides the Metropolitan Farm. The River Yarra and other rivers and also many large drains flow into the bay, and their effects must be considered. For this reason it was decided to examine water samples from the Yarra and various large drains around St. Kilda and Port Melbourne, including the Elwood Canal, Shakespeare-grove drain, St. Kilda, the Princes-street and Cruickshank-street drains, Port Melbourne.

All water samples were collected at or just below the surface since the number of bacteria here is generally found to be greatest.

IV. Results of Examination of Water Samples.

1. *Bacterial Content of Ocean Water from Bass Strait.*—Altogether eleven samples of ocean water were examined. Three were received on 12.5.32, two on 16.6.32, three on 21.7.32, and three on 20.8.32. They were numbered consecutively from 1 to 11. The plate counts on samples 1 to 8 inclusive were carried out by three methods, using agar incubated at 37° C. and 22° C. and gelatine incubated at 22° C. Table 1 shows the results of these counts.

TABLE 1.

Total Number of Aerobic Organisms in 1 c.c. of Ocean Water.

Sample Number.	Agar at 37° C.	Agar at 22° C.	Gelatine at 22° C.
1	31	11	54
2	18	7	53
3	2	1	37
4	11	14	28
5	9	9	33
6	15	393	829
7	5	14	100
8	18	67	523

Numbers 6 and 8 are remarkable in that they show a very much higher count on the plates incubated at 22° C. than any of the other samples. The figures for gelatine are conspicuously greater than for the others. The low figures for agar at 37° C. seem to indicate that there are only a few organisms in the ocean which can grow at this temperature. Taking the figures as a whole the bacterial content of uncontaminated sea water enumerated in this way is very low, and is lower than that of a good drinking water.

Samples 9, 10, and 11 were plated only on gelatine, but the plates were done in duplicate. The results are shown in Table 2.

TABLE 2.

Total Number of Aerobic Organisms in 1 *c.c. of Ocean Water.*

Sample No.	Gelatine at 22° C.	Average.
9	8, 7	8
10	12, 13	13
11	20, 10	15

As in the previous samples these figures are extremely low.

The total bacterial content being so low, it was unlikely that *B. coli* would be present in large numbers; therefore, in carrying out the presumptive test for this organism on all samples, 100 c.c. divided into ten equal portions were used. This range allowed the determination in all these samples of the smallest quantity of water containing *B. coli.* The results of these presumptive tests are shown in Table 3.

TABLE 3.

Presumptive Test for the Presence of B. coli *in Ocean Water.*

Sample No.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.
1	+	—	—	—	—	—	—	—	—	—
2	+	—	—	—	—	—	—	—	—	—
3	+	—	—	—	—	—	—	—	—	—
4	+ A	+ B	—	—	—	—	—	—	—	—
5	+	—	—	—	—	—	—	—	—	—
6	+	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
8	+ A	+ B	+ C	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—

These results showed that a reaction which may be due to the presence of *B. coli* was indicated in samples 1, 2, 3, 5, 6 in 100 c.c., in sample 4 in 50 cc., in sample 8 in 30 c.c., and in samples 7, 9, 10, and 11, no *B. coli* in 100 c.c.

It may be seen, however, from Table 4 that confirmatory tests, performed with three pure cultures, *a, b,* and *c,* from MacConkey plates for each positive presumptive tube, proved these reactions with one exception to be due not to true *B. coli,* but to other lactose fermenting organisms. Where more than one positive tube was obtained from one sample, the tubes have been distinguished in Tables 3 and 4 by the letters A, B, C.

TABLE 4.

Results of Confirmatory Tests for B. coli *in Ocean Water.*

Sample No.		N.R.L.	Citrate.	Indol.	Methyl Red.	Conclusion.
1	(*a*)	+	+	−	−	Not *B. coli*
	(*b*)	+	+	−	−	
	(*c*)	+	+	−	−	
2	(*a*)	+	+	−	−	
	(*b*)	+	+	−	−	
	(*c*)	+	+	−	−	
3	(*a*)	+	−	−	+	*B. coli*, but Indol negative
	(*b*)	+	−	−	+	
	(*c*)	+	−	−	+	
4A	(*a*)	+	+	−	−	Not *B. coli*
	(*b*)	+	+	−	−	
	(*c*)	+	+	−	−	
4B	(*a*)	+	+	−	−	
	(*b*)	+	+	−	−	
	(*c*)	+	+	−	−	
5	(*a*)	+	+	−	−	
	(*b*)	+	+	−	−	
	(*c*)	+	+	−	−	
6	..	No cultures as no growth on MacConkey plates.				
8A	(*a*)	+ after 4 days incubation	+	−	+	*B. coli* should produce acid and gas in neutral red lactose in 24 hours, four days being too long a period. These cultures are also citrate + and it is concluded, in spite of the + methyl red reaction, that they are NOT *B. coli*.
	(*b*)	+	+	−	+	
	(*c*)	+	+	−	+	
8B	(*a*)	+	+	−	+	
	(*b*)	+	+	−	+	
	(*c*)	+	+	−	+	
8C	(*a*)	+	+	−	+	
	(*b*)	+	+	−	+	
	(*c*)	+	+	−	+	

The conclusions from this section of the work are that ocean water in the vicinity of Victoria has a very low bacterial content compared with most other natural waters. Generally, it does not contain *B. coli* in less than 100 c.c., and only rarely in this volume, which is quite in accord with the results of an examination of deep sea water carried out by the British Royal Commission for Sewage Disposal, and described in the report for 1904(11).

2. *Bacterial Content of Bay Water.*

The first trip was made on 8.7.32 on the s.s. *Sphene*, when a strong southerly breeze was blowing, and fifteen samples were collected. The thick line on Fig. 1 indicates the course, and the following sampling points:—

1. River Yarra below Salt Water River.
2. Yarra Entrance.
3. Gellibrand Lighthouse.
4. Off Point Cook.
5. Near Werribee River. See Map Location.
6. Near Werribee River. See Map Location.
7. Near Werribee River. See Map Location.
8. Off Metropolitan Farm Jetty.
9. See Map Location.
10. Off Little River.
11. See Map Location.
12. Steam Boat Buoy.
13. Outer Harbour, Geelong.
14. Inner Harbour, Geelong.
15. Geelong Wharf.

The total bacterial count for these samples is indicated in Table 5.

TABLE 5.

Total Number of Aerobic Organisms in 1 c.c. of Bay Water, 8.7.32.

Sample No.	Agar at 37° C.	Agar at 22° C.	Gelatine at 22° C.
1	1,000	10,000	Innumerable
2	1,000	9,000	Innumerable
3	17	300	10,000
4	5	45	..
5	2	5	45
6	1	4	72
7	1	0	0
8	1	2	91
9	3	30	115
10	6	21	32
11	0	9	45
12	2	10	83
13	7	54	183
14	29	41	625
15	62	94	Innumerable

Reference to Table 5 and the locations of sampling points will show that the high counts in samples 1-3 are due to the fact that they were taken from the River Yarra or from points in the bay near its mouth. Samples 4-13 taken from the Gellibrand Lighthouse to Outer Harbour, Geelong, show counts comparable with those of ocean water. This result is most important, because most of these samples were taken opposite the farm, about 2 miles from the shore. Samples 14 and 15, taken close to Geelong, once more show a high count, which is no doubt due to local pollution.

TABLE 6.

Results of Presumptive Tests for B. coli *in Bay Water.*

No.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	10 c.c.	1 c.c.	0·1 c.c.	0·01 c.c.	0·001 c.c.
1	+	+	+	+	+	+	+	—	—
2	+	+	+	+	+	+	+	—	+
3	+	+	—	—	—	—	—	..	..
4	+	—	—	—	—	—	—	..	..
5	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—
7	+	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—
10	+	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—
14	+	+	+	+	+	+	—	..	..
15	+	+	+	+	+	+	—	..	..

It will also be seen from Table 6 that the results for the presumptive test for *B. coli* correspond with those for the total bacterial count; and the fact that "positive" reactions were obtained in such small quantities of samples 1, 2, 3, 14, and 15 may be explained in the same way, namely, on account of their location.

When further confirmatory tests were applied to determine the identity of the organisms producing the reactions it was found that true *B. coli* was present in the water around Melbourne and Geelong in quantities varying from 1 c.c. to 10 c.c., but samples collected opposite the farm failed to show the presence of true *B. coli* in 50 c.c., and were therefore considered as pure as ocean water samples.

Another trip was made on the s.s. *Edina* on 10.11.32. A fresh to strong northerly breeze was blowing, and the course pursued, shown as a fine line in Fig. 1, was almost identical with that taken by the s.s. *Sphene* on 8.7.32.

The estimation of the total bacterial content and the presumptive and confirmatory tests for *B. coli* were performed in exactly the same way as for the samples taken on the s.s. *Sphene*. Identical results were obtained from these *Edina* samples, which showed a low bacterial content in samples 3 to 14 with a correspondingly low *B. coli* figure, this organism being absent from 50 c.c. of each of these samples. Contamination was again evident in the River Yarra, but the samples collected near Geelong were of good quality.

A third trip to Geelong was made on the s.s. *Edina* on 9.8.32, when there was a strong south-west breeze and the course is shown in Fig. 1 as a dotted line. The locations of the sampling points on this occasion were at a much greater distance from the farm. Most samples were again as free from contamination as ocean water, while those from the vicinity of the Yarra and the Geelong wharf showed definite evidence of pollution when subjected to all tests.

Summarizing the conclusions from these three trips, it may be said that under different weather conditions no trace of pollution of the waters of the bay can be found in the neighbourhood of the Metropolitan Farm at a minimum distance of slightly under 2 miles from its shore. The waters of the River Yarra near Victoria Dock are always polluted to some extent, *B. coli* being present in 0.1 c.c., and often in 0.01 c.c. of the water. For a river such as the Yarra at this point, this is not an objectionable degree of pollution, and its effect is not far-reaching, as there is no trace of pollution beyond the Gellibrand Lighthouse. There appears to be some pollution of local origin around the Geelong wharf, as the water at the entrance to Corio Bay is as pure as ocean water.

3. *Bacterial Content of Bay Water in the Immediate Neighbourhood of the Metropolitan Farm, Werribee.*

The sea water samples collected from the locations just off the foreshore of the Metropolitan Farm near Werribee, and marked by the three series of buoys A, B, and C (Fig. 2), were subjected to the same procedure as that used for bay water for the determination of the *B. coli* content, presumptive positive results in neutral red lactose broth always being checked by confirmatory tests as outlined above.

The results given in Table 7 of tests on samples collected during the first five trips to Werribee show a concentration of *B. coli* round A7, B7, and C9, which are the most easterly stations. This seemed to indicate, as the chlorine figures also do, that there is a natural easterly drift. For this reason a D series of buoys was set up farther to the east to try to trace any further pollution in this direction. A second series of

five trips was made for the collection of samples from these new positions as well as from the original A, B, and C series, and from the six large effluent drains. The results of the *B. coli* tests for these samples are also given in Table 7, and again show no evidence of pollution along the extended line 800 yards from the shore. Chemical tests were performed on all these samples, and the complete set of dilution factors from the chlorine determinations for the ten trips is listed in Table 9.

TABLE 7.

B. coli *content of Sea Water at the Buoys near the Metropolitan Farm.*

—	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
C1	+50*	−50	+50	+ 0·1	+25	+25	−50	−50	−50	..
C2	−50	−50	−50	+ 1	+25	+ 1	−50	−50	−50	..
C3	+25	+25	+ 1	−50	−50	−50	−50	−50	−50	..
C4	+20	+50	+ 1	−50	+20	−50	−50	−50	−50	..
C5	+50	+50	+ 0·1	−50	−50	−50	−50	−50	−50	..
C6	+10	+25	+ 1	−50	−50	−50	−50	−50	−50	+12
C7	+ 1	−50	+ 0·1	−50	−50	+25	+50	−50	−50	..
C8	+25	+12	+ 0·1	−50	−50	−50	+10	−50	−50	+10
C9	+ 0·1	+ 0·01	+ 0·01	− 1	− 1	+ 1	+ 1	− 1	− 1	+ 1
B1	− 1	+ 1	+ 1	+ 1	− 1	+ 1	− 1	− 1	− 1	..
B2	− 1	+ 0·1	− 1	+ 0·1	− 1	− 1	− 1	− 1	− 1	..
B3	+ 1	+ 0·1	+ 1	− 1	− 1	+ 0·1	+ 1	− 1	− 1	..
B4	+ 1	+ 0·1	+ 0·01	− 1	− 1	− 1	+ 1	− 1	− 1	..
B5	+ 1	+ 0·1	+ 1	+ 1	+ 1	− 1	+ 0·1	− 1	− 1	..
B6	+ 1	− 1	+ 0·1	− 1	− 1	− 1	− 1	− 1	− 1	+ 0·1
B7	+ 0·01	+ 0·001	+ 0·1	− 1	− 1	+ 1	+ 1	− 1	− 1	+ 0·1
A1	− 1	+ 0·1	+ 0·1	+ 0·1	+ 1	+ 0·01	+ 1	− 1	− 1	..
A2	+ 0·01	+ 0·01	+ 0·01	+ 0·1	+ 1	+ 0·001	+ 0·1	− 1	− 1	..
A3	+ 0·01	+ 0·01	+ 0·001	+ 0·1	+ 0·01	− 1	+ 1	− 1	− 1	..
A4	+ 1	+ 0·01	+ 0·01	+ 0·1	+ 0·01	+ 1	+ 0·1	− 1	− 1	..
A5	+ 1	+ 0·1	+ 0·1	+ 1	− 1	+ 1	+ 0·1	− 1	− 1	..
A6	+ 0·1	+ 0·01	+ 0·1	− 1	− 1	− 1	+ 0·01	− 1	− 1	+ 0·1
A7	+ 0·01	+ 0·01	+ 0·01	− 1	− 1	+ 0·01	+ 0·01	− 1	− 1	+ 0·1
D1	..	..	..	..	..	+ 1	− 1	− 1	− 1	−50
D2	..	..	..	..	..	+ 0·1	+ 0·1	− 1	− 1	−50
D3	..	..	..	..	..	+ 1	+ 0·1	−50	−50	−50
D4	..	..	..	..	..	+50	+10	−50	−50	−50
D5	..	..	..	..	..	+ 0·01	− 1	− 1	− 1	−50
D6	..	..	..	..	..	+ 0·01	+ 1	− 1	−50	−50
D7	..	..	..	..	..	+ 1	−50	−50	−50	−50

* All numbers are in terms of c.c.

— in table indicates that *B. coli* is absent in the volume indicated and that no larger volume was tested.

\+ in table indicates that *B. coli* is present in the volume indicated but not in any smaller volume.

I. Samples for 6.9.32 with west to south-west wind. Easterly drift parallel with shore.

II. Samples for 15.9.32 with slight south-west wind and slight westerly drift. Sea almost dead calm.

III. Samples for 21.9.32 with squally southerly wind. For half mile out sea turbid.

IV. Samples for 28.9.32 with south-westerly wind. North-westerly drift with incoming tide. Water fairly clear.

V. Samples for 4.10.32 with strong northerly wind. North-westerly drift with incoming tide, sea slightly turbid.

VI. Samples for 18.10.32 with mild south breeze till noon, northerly wind till 3 p.m. then strong south-westerly. Sea very calm and clear till 3 p.m., then rough. Incoming tide and easterly drift. Marked discolouration extending easterly from D5, near mouth of Werribee River.

VII. Samples for 28.10.32 with squally south-westerly wind. Sea very rough. Easterly drift.

VIII. Samples for 8.11.32 with southerly to south-easterly wind, very light in morning, stronger in afternoon. Very slight south-westerly drift. Sea very clear.

IX. Samples for 15.11.32 with southerly wind, north-westerly drift, incoming tide.

X. Samples for 22.11.32 with strong north-westerly to westerly wind and easterly drift. Sea clear and slightly rough. A south-westerly squall arose, and no further samples could be collected after 11.45 a.m.

It may be seen from the data in Table 7 that the A and B series of samples show definite evidence of pollution, *B. coli* generally being present in quantities varying from 1 c.c. to 0.001 c.c. This result was to be expected on account of the close proximity of these sampling points to the shore. The C samples, however, taken at a greater distance (800 yards), compare favorably with ocean water, in many cases no *B. coli* being present in 50 c.c.

The low *B. coli* content of sea water in the vicinity of the farm as shown by the results of Table 7 is striking. Almost always the C samples compare favorably with ocean water, showing no *B. coli* in 50 c.c. This is better than a good drinking water. On 18.10.32 and 28.10.32 the *B. coli* figure for the drain effluents was high, showing on an average 10,000 to 100,000 organisms in 1 c.c. (Table 8), and consequently some

TABLE 8.

B. coli *content of Effluents.*

No.	Date of Tests.					
	6.9.32.	18.10.32.	28.10.32.	8.11.32.	15.11.32.	22.11.32.
95E ..	+0·0001	+0·0001	+0·00001	+0·001	−0·1	+0·001
55E ..	+0·0001	+0·0001	+0·00001	+0·001	+0·01	+0·0001
15E ..	+0·0002	+0·00001	+0·001	+0·00001	+0·01	..
25W ..	+0·001	+0·0001	+0·00001	+0·001	+0·001	..
105W ..	+0·0002	+0·0001	+0·0001	+0·01	+0·0001	..
165W ..	+0·0001	+0·0001	+0·0001	+0·01	+0·001	..
Crude Sewage ..	+0·000001	..	..	..	..	..

of the A samples were heavily polluted (*B. coli* "positive" in 0.01 c.c. and even in 0.001 c.c.). In spite of this the B samples only occasionally show *B. coli* present in 1 c.c., and the C samples, as stated above, could be considered unpolluted. The D samples

showed round the farm jetty a small amount of pollution, which seems to disappear just beyond the end of the jetty. On 18.10.32 D5, D6, and D7 showed quite a marked degree of pollution presumably due to the Werribee River which was enormously swollen by recent heavy rains. This river does not receive drainage from the farm. The discolouration due to the river water extended in an easterly direction, and provided further evidence of a natural easterly drift. Under normal conditions samples from these three stations showed no evidence of pollution. The particularly good results from sea water obtained on 8.11.32 and 15.11.32 may be correlated with the very low *B. coli* content of the effluent drains on these days. (See Table 8.)

TABLE 9.

Dilution of Effluents by Sea Water near the Metropolitan Farm, Werribee.

Number of volumes of Sea Water per one volume of Farm Effluent estimated from determinations of chloride radicle.

Sample No.	Date of Tests									
	6.9.32.	15.9.35.	21.9.32	28.9.32.	4.10.32.	18.10.32.	28.10.32.	8.11.32.	15.11.32.	22.11.23.
C1 ..	100	100	100	100	100	100	100	100	100	..
C2 ..	100	100	100	100	100	100	100	100	100	..
C3 ..	100	80	100	100	100	100	100	100	90	..
C4 ..	100	50	100	100	100	70	100	100	100	..
C5 ..	95	100	100	95	60	100	100	100	80	..
C6 ..	100	100	95	80	60	100	100	100	100	100
C7 ..	40	100	100	60	60	100	100	100	100	..
C8 ..	60	60	100	60	60	100	90	100	90	..
C9 ..	20	8	40	18	50	20	100	100	30	100
B1 ..	100	50	95	100	100	100	100	60	25	..
B2 ..	100	12	100	60	100	100	100	60	90	..
B3 ..	80	10	100	100	100	40	100	100	60	..
B4 ..	80	8	100	100	40	100	90	100	90	..
B5 ..	100	27	80	95	40	100	100	100	80	..
B6 ..	40	60	95	40	50	100	35	100	100	100
B7 ..	27	12	60	10	40	30	100	100	35	90
A1 ..	100	40	100	100	100	40	100	40	100	..
A2 ..	40	7	8	50	27	6	100	35	30	..
A3 ..	23	7	26	27	20	16	100	60	25	..
A4 ..	60	8	40	40	15	8	60	100	40	..
A5 ..	60	14	80	50	40	14	30	100	35	..
A6 ..	20	14	60	13	40	100	100	100	35	100
A7 ..	18	9	11	11	26	6	9	30	16	100
D1 ..	..	..	..	..	..	25	35	30	25	100
D2 ..	..	..	..	..	..	40	100	100	90	100
D3 ..	..	..	..	..	..	100	100	100	100	100
D4 ..	..	..	..	..	..	100	100	100	100	100
D5 ..	..	..	..	..	..	..	..	40	60	100
D6 ..	..	..	..	..	..	20	100	100	80	100
D7 ..	..	..	..	..	..	50	100	100	100	..

The diluting effect of the sea water on effluents was further determined by obtaining the chlorine content of duplicate samples. These figures are given in Table 9, and were kindly furnished by Mr. V. G. Anderson, advising chemist to the Board, who has conducted an extended chemical survey in the area discussed in this report. A and B samples showed a lower

chlorine content than sea water, demonstrating the fact that at these distances the fresh water of the effluents, containing only a very small amount of chloride, constituted a definite proportion of the samples. The C samples on the other hand had practically the same chlorine content as sea water, the fresh water of the effluents at this distance being so diluted as to be almost undetectable. This chemical evidence correlates very well with the evidence obtained from the *B. coli* tests.

Summarizing the results of the ten investigations, it can be said that even during wet spring months when the land must be worked to its utmost capacity, because its absorbing power is low, the pollution of the sea, though fairly marked within 100 yards of the shore, altogether disappears within 800 yards; and that this pollution along the shore does not extend westward past Little River, nor eastward past the farm jetty. A graphic representation of this result is shown in Fig. 2, where the broken dotted line indicates the boundary of the average area of bacterial pollution. It will be noticed that this area corresponds very well with that given by the chlorine figures, indicating the dilution, shown in Fig. 2 by a broken line. The summer effluents, which come only from specially selected areas and are high grade, have no noticeable polluting effects on the sea at all. It is of interest to note that similar investigations of the efficiency of sea water dilution methods have been carried out at Rothesay, Scotland(4), and at Newcastle, New South Wales(10). In the case of the former there was no pollution of the beach, whereas with the latter samples taken close to the beach, which was used by bathers, showed a high *B. coli* content, and the pollution was considered sufficient to endanger public health.

4. *Examination of Effluents from City Drains discharging into the Bay.*

On several occasions water from the mouths of various drains round St. Kilda and Port Melbourne was tested for the presence of *B. coli* and total bacterial content. Water from the Yarra, apart from that collected on the various bay trips, was also examined. Some results are shown in Table 10.

The large bacterial count of many of these drains by all three methods should be noticed. In every examination of drain or river water green fluorescent bacteria grew up on all the plates, though these were never isolated from sea water. These organisms of the type of *Pseudomonas* appear to be natural inhabitants, almost constant in polluted fresh water. The Princes-street drain, as judged from the one sample examined from it, was the worst of all these drains. The Elwood canal and the Shakespeare-grove drains were also highly polluted, and although these three drains had a fairly high *B. coli* content,

it was not so high as that of the farm effluents. As a consequence the drains mentioned above will probably have no detectable polluting effects on the bay water as they discharge only a small volume which will be very quickly diluted.

TABLE 10.

Results of Bacteriological Examination of Drain Effluents.

Drain.	Date.	Total Bacterial Content in thousands per c.c.			*B. coli* Content. in c.c.
		Agar 37° C.	Agar 22° C.	Gel. 22° C.	
Elwood Canal	8.6.32	7	9	9	+ 0·1 or less
	25.7.32	7	20	*	+ 0·01
					+ 0·01
Shakespeare-grove Drain	8.6.32	3	3	6	+ 0·1 or less
	30.6.32	2	2	*	+ 0·01
	25.7.32	1	3	4	+ 0·1
Princes-street	25.7.32	4	5	10	+ 0·001
Cruickshank-street ..	25.7.32	2	3	7	+ 1
Lagoon Dock	8.6.32	0·5	1	4	+25
	25.7.32	0·4	5	11	+ 1
Yarra (Spencer-street) ..	30.6.32	0·8	9	10	+ 0·1 or less
Yarra (Williamstown Ferry)	25.7.32	1·3	3	9	+ 0·01

* Indicates that the plates contained innumerable colonies.

V. General Summary and Conclusions.

The investigation described in this report had as its object the detection of the degree of pollution, if any, of the waters of Port Phillip Bay by the effluents from the Metropolitan Sewage Farm, Werribee. The amount of pollution of the various water samples examined was judged by their *B. coli* content as compared with that of ocean water collected in Bass Strait. Samples of sea water from different points in the neighbourhood of the farm and between Melbourne and Geelong were examined, and the bacterial content of various drains emptying into the bay was determined.

Water samples were collected from the open ocean and inside Port Phillip Bay between Melbourne and Geelong, those coming from the immediate vicinity of the farm being collected about 2 miles from the shore. Under various weather conditions all the samples from inside the bay with the exception of those taken in the River Yarra and at the Geelong wharf were as pure as ocean water. This established the fact that there was no pollution of the water 2 miles from the farm coast line.

Samples were also collected from the front of the farm close in shore, at definitely located points, during various conditions of weather, tide, and drift. Analyses of these samples showed that in winter, when the effluents contain large numbers of organisms, all trace of pollution is lost within 800 yards of the shore, while at 400 yards from the shore it is very slight. With summer effluents no trace of pollution is detectable at all in the amounts tested, even at the points nearest to the shore. Chlorine figures for the samples showed that the *B. coli* figure closely followed the dilution factor, the former decreasing as the latter increased.

No trace of pollution was ever found in a westerly direction from the farm, so that there is no likelihood of contamination of the water of that part of the bay extending towards Geelong. In an easterly direction all trace of pollution disappears just past the farm jetty. During the period of this investigation adequate dilution of the effluents occurred during all weathers.

The various city drains which were tested do not show any very marked power of pollution, nor does the River Yarra, considering its uses. In all cases the dilution factor should be sufficient to remove all traces of pollution immediately.

Points of bacteriological interest noted were the almost complete correlation of the citrate and methyl red tests and the possible use, therefore, of the citrate test alone as confirmatory for *B. coli*, using citrate agar cultures incubated for a period of only 24 hours. The indol test did not agree very well with the citrate or metyl red tests.

The general conclusion to which the results of this investigation leads, is that the Melbourne method of sewage treatment by broad irrigation followed by the dilution of the effluents in the sea, is successful from the point of view of the public amenities, and most important, the safety of public health. During the period of this investigation the effluents from the Metropolitan Farm did not cause serious or extensive pollution of the waters of Port Phillip Bay, and their effect was only evident in a very small area extending not more than 800 yards from the shore from Little River on the west to the farm jetty on the east.

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